



SCIENTIFIC GROUP ON ORAL ENTERIC VACCINES

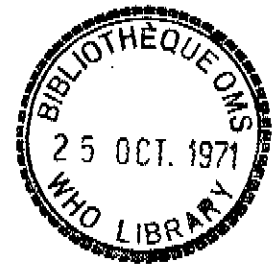
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EXPERIMENTAL MODELS FOR ESCHERICHIA COLI INFECTIONS  
AND IMMUNITY STUDIES

by

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Several obstacles obstruct the approach to the study of the etiology of infectious diseases of the alimentary tract. One is the presence of large numbers of actively multiplying bacteria of many kinds. This is a particularly formidable handicap when the organisms suspected of being involved in disease processes belong to species that are normal inhabitants of the alimentary tract, e.g. Escherichia coli. It necessitates the use of bacteriological techniques that are both quantitative and qualitative. An important complicating factor in the quantitative approach is the rapidity with which bacteria proliferate in some regions of the alimentary tract after death. For example, the concentration of viable E. coli organisms in the chyme in the small intestine of calves may increase 10-fold within three hours and 1000-fold within six hours of being killed (Smith, 1962). Also, marked macroscopic and microscopic changes in the alimentary tract occur shortly after death, some being perceptible within one and a half hours.

It is apparent then that the amount of reliable information on the alimentary tract that can be obtained from examinations performed some time after death is seriously limited. This, together with the fact that the examination of faecal material sometimes gives little indication of the bacteriological state of the small intestine, emphasizes the great difficulties of studying many of the important aspects of diseases such as Escherichia coli diarrhoea in human beings. In fact, it becomes immediately obvious that if progress is to be made in solving many of the problems of E. coli diarrhoea a suitable animal model is essential.

E. coli infections of the alimentary tract occur naturally in some species of domestic animal, including pigs, calves and lambs. As far as can be ascertained, the disease in these animals closely resembles that in babies, but with one important difference - the serotypes of E. coli that cause the disease in animals are different from those that cause the disease in babies. Consequently, for an experimental model, one may use domestic animals infected with serotypes that naturally cause disease in them. Alternatively, one may prefer to try to set up suitable infections with human serotypes in laboratory animals, animals which, apparently, do not suffer from E. coli diarrhoea naturally.

While reports have been published on the successful establishment of serotypes pathogenic to man in the alimentary tract of laboratory animals, clinical disease does not usually result. Recently, however, Mushin, Ford & Hughes (1970) have produced clinical disease

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in suckling NCS mice nine to 12 days old, by giving them enteropathogenic human strains by mouth. Unfortunately, the disease produced does not resemble that seen in babies in that the mice show no sign of diarrhoea, the essential lesion being a bacteraemia.

The accumulating evidence from a number of research centres in recent years strongly supports the view that, at the present time, the pig infected with its natural E. coli enteropathogens constitutes the most suitable experimental model for elucidating many of the problems of E. coli diarrhoea. An additional incentive for using this particular model is that E. coli is considered to be the most important single cause of loss due to disease in the pig industry. Some of the more important parts of this evidence are summarized below.

Under natural conditions, E. coli produces in piglets a severe cholera-like disease during the first few days of life. Diarrhoea is extremely severe and intense dehydration may be present within a few hours of the onset of the disease. Death may follow within one or two days. The pig is unique among domestic animals in that E. coli infection may also occur in the more immediate post-weaning period, weaning normally taking place when pigs are six to eight weeks old. Here, it manifests itself in one of two forms. One form is severe diarrhoea resembling that seen in piglets, and the other is oedema disease, which is characterised by locomotor ataxia due to involvement of the central nervous system and by the accumulation of oedematous fluid in the organs, notably in the gastric mucosa; these signs of oedema disease are thought to be the result of the absorption from the intestine of E. coli metabolites.

Pigs belonging to litters suffering from different forms of natural E. coli infection can be sacrificed at various stages of the disease and submitted to immediate bacteriological examination. The results can then be compared with those of their healthy litter-mates. The bacteriological picture in both pigs and piglets suffering from diarrhoea is the same, and consists of an enormous proliferation in the anterior small intestine of E. coli organisms belonging to specific serotypes, the concentration of organisms in this region often being 10 000 times higher than that found in healthy animals. Although small numbers of E. coli may occasionally be found in the internal organs of animals in the terminal stages of infection, there is no evidence of invasion at other times. The concentrations in the chyme of other organisms that normally inhabit the alimentary tract remain largely unaltered. High concentrations of the specific serotypes of E. coli are also found in the anterior small intestine of pigs in the early stages of oedema disease. By the time the characteristic clinical and pathological signs are present, the concentrations, however, may have become quite low.

Neonatal diarrhoea identical with the natural disease can be produced in the first few days of life in suckling piglets by the oral administration of strains of E. coli belonging to those serotypes known to be enteropathogenic for pigs. The usual sow litter size of eight to 12 piglets is adequate for the performance of many kinds of experiment. The experimental disease can be set up with a reasonable degree of uniformity in the litters of primiparous sows. We sometimes experience difficulty with the litters of multiparous animals, presumably because of the higher antibody content of their colostrum. This can be overcome, if necessary, by removing the young from their mothers immediately they are born and rearing them artificially, a reasonably simple procedure. Since trans-placental immunity does not take place in the pig, such piglets are pre-eminently suitable for immunity studies because they will be practically devoid of antibody. In some laboratories, gnotobiotic piglets are used for these and other studies.

Most workers have not been successful in producing clinical disease in pigs during the immediate post-weaning period by the oral administration of E. coli cultures. We have 'in-bred' pigs obtained from a farm where post-weaning diarrhoea and oedema disease was a common occurrence and, as a result, have established a herd in which these diseases can be produced with a high degree of frequency. For the last few years, we have used piglets and recently-weaned pigs from this herd for studying experimental E. coli infections with satisfactory results.

### Ligated intestine test

Although rabbit ligated intestine is suitable for studying the enterotoxin of Vibrio cholerae, most investigators have found it rather unreliable for identifying enterotoxigenic strains of E. coli, irrespective of their origin. This is particularly the case when cultures are tested, a considerable proportion of those strongly suspected of being enteropathogenic either causing dilatation inconsistently or not at all. A greater degree of success in rabbits was obtained by Smith & Gyles (1970b) using filtrates of lysed cultures, their so-called LT preparations, instead of live cultures. The dilating substance (enterotoxin) was neutralized by antiserum either in a serotype-specific or strain-specific manner. However, preparations from a few human strains belonging to serotypes generally considered to be enteropathogenic consistently failed to cause dilatation while those of some non-pathogens did. Thus, even when these LT preparations are used, one has to conclude that either the enterotoxigenicity, and hence the enteropathogenicity, of some human serotypes or the reliability of the ligated rabbit intestine is in doubt.

In contrast to the rabbit, the pig has been found by all investigators to be a reliable animal for use in ligated intestine tests, particularly when live cultures themselves are injected into the intestinal segments. It can only be used for testing pig strains of E. coli; human, bovine and ovine enteropathogenic strains, and cell-free preparations of cultures of these strains, do not dilate pig intestine. A few strains of E. coli that cause diarrhoea in piglets dilate piglet, but not pig, intestine and, consequently, have to be studied in piglets (Moon & Whipp, 1970). One additional advantage, other than reliability, of pigs over rabbits is that as many as 50 to 60 strains can be tested in one pig.

Studies on cell-free preparations in ligated pig intestine have revealed that, in artificial culture, the enterotoxin of porcine strains exists in two forms, a heat-labile antigenic form, termed LT, and a heat-stable non-antigenic form, termed ST. All enteropathogenic strains produce the ST form but only those possessing the K88 antigen produce detectable amounts of the LT form.

Whether the substance produced by human strains of E. coli and causing dilatation of ligated rabbit intestine is indeed the substance that causes diarrhoea in babies is, of course, open to question. As far as porcine strains are concerned, the relevance of positive reactions in ligated rabbit intestine can be, and has been, assessed by performing similar experiments in pigs, by giving cell-free culture fluids to piglets and finally by giving piglets live cultures of the strains of E. coli in question. This type of approach is largely impossible to pursue in the case of human strains.

The discovery that the enterotoxin of porcine enteropathogenic strains can exist in two forms was facilitated by the fact that it is controlled by a transmissible plasmid termed Ent (Smith & Gyles, 1970a). By giving piglets, by mouth, materials from strains that differed from each other solely by the presence or absence of Ent, it was possible to strengthen the evidence that enterotoxin itself, and not other bacterial products, was the principal cause of the diarrhoea produced in the piglets. More recently, we have demonstrated the transmissible nature of the genetic elements controlling enterotoxin production in an O26:K60 human strain and in some calf and lamb enteropathogenic strains, indicating that it is controlled in the same manner as is the porcine enterotoxin. It is noteworthy, too, that not only will Vibrio cholerae enterotoxin dilate ligated pig intestine but that an antigenic relationship exists between this enterotoxin and that of porcine strains of E. coli (Gyles & Barnum, 1969). Thus, although certain differences exist between the enterotoxins of enteropathogenic strains of E. coli of different origins and of Vibrio cholerae, they also have similarities, which leads one to the tentative conclusion that they may all be based on the same toxic molecule.

One of the strongest arguments in favour of using the pig E. coli experimental model is the fact that three important characteristics of most porcine enteropathogenic strains of E. coli are known to be plasmid-controlled. These are the production of enterotoxin, haemolysin and K88 antigen. By plasmid transmission and by the selective removal of plasmids with cytoplasmic poisons ('curing'), strains of E. coli that differ only in regard to the particular combination of these plasmids possessed can be obtained. We have used such strains in infection experiments in pigs. So far, we have been unable to show that the haemolysin plays any part in the pathogenesis of E. coli infection. The K88 antigen, however, is importantly involved in that it permits organisms possessing it to proliferate in the anterior small intestine, apparently because the antigen facilitates their adhesion to the epithelium. The possession of the enterotoxin plasmid, in addition to the K88 one, is largely responsible for the severity of the diarrhoea that follows proliferation in the small intestine. By plasmid transmission, we were able to convert a non-pathogenic strain of E. coli into an enteropathogenic one in that it produced diarrhoea when given to suckling piglets. It would be surprising if transmissible plasmids cannot be exploited much further in solving many of the problems of E. coli infection.

The pig can also be used to study immunity in E. coli diarrhoea. We have given pigs in the immediate post-weaning period antiserum parentally and then infected them orally. A definite protective effect against diarrhoea was obtained provided the antiserum used had been prepared against the infecting strain, i.e. it was of a specific character. All the evidence pointed to the protection being anti-bacterial rather than anti-enterotoxic. Antisera provided an additional type of protection against oedema disease. This was neither strain-specific nor anti-bacterial in nature. It appeared to be directed against the actual E. coli metabolites that caused the pathological changes characteristic of oedema disease.

Piglets removed from their mothers at birth and reared artificially are more satisfactory than weaned pigs for studying passive immunity to E. coli infection because, as stated previously, they are almost completely devoid of immuno-globulins; piglets normally acquire these substances via colostrum. Such animals can be given antisera by mouth or parentally, before they are given cultures of E. coli. The course of the subsequent infection can then be assessed clinically and bacteriologically. Gnotobiotic piglets have been used fairly extensively in these studies, particularly by Kohler and his colleagues (for reference, see Kohler & Cross, 1971). These workers and Miniats, Mitchell & Barnum (1970) observed that antisera had a definite protective effect against infection with homologous organisms. They were of the opinion that the activity of the antisera was directed principally against the enterotoxin because there was no observable difference in the number of infecting organisms in the alimentary tract of piglets protected by antisera than was the case in infected control piglets. We have used conventionally-born piglets which acquired, or were given, bacteria additional to the infecting enteropathogenic strain. In these piglets, antisera, particularly when given by mouth, had a pronounced antibacterial effect.

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